to compensate by making the dilatometer bulb slightly longer.

If the dimensions were chosen so as to make the fat sample smaller, one could reduce the capillary bore in order to maintain the present sensitivity of the dilatometer, which is now about 2.8 and 1.8 mm./1% "Solids Content" for a 9- and 6-g. sample, respectively. The diameter of the capillary bore is well over 2 mm. so there seems to be room for such a reduction.

Summary

A change was made in the dilatometer design, allowing a substantial reduction in the time required to complete a "Solids Content" determination in edible fats.

The modified dilatometer has a hollow stopper that extends nearly to the bottom of the dilatometer bulb, thereby giving the fat sample the shape of a hollow cylinder. The surface area effecting heat transfer is increased, and the thickness of the fat sample is reduced, resulting in a considerable reduction in the time required to obtain temperature equilibrium throughout the fat sample.

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The Antioxidants of the Osage Orange Fruit[1]

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AMONG the natural materials which have been shown to contain potent antioxidants for fats is the mature fruit of the osage orange (*Maclura pomifera* [Raf.] Schneider). Initial work in this laboratory (3) showed that carbon tetrachloride extracts of the powdered dried fruit increased the keeping time of lard by more than one hundredfold; non-crystalline allophonate and acetyl derivatives prepared from the extract were inactive but became active antioxidants after hydrolysis.

In subsequent work (2) osajin and pomiferin were isolated from the extracts. Tests with the pure compounds showed osajin only slightly active while pomiferin accounted for a large part of the total activity. However a non-crystalline fraction contained a synergistic substance. Clopton (1) reached similar conclusions apparently from studies with concentrates since no tests with pure pomiferin were presented.

This paper describes the chromatographic separation of antioxidants from the osage orange extracts. Besides osajin and pomiferin, each of which constitutes 3 to 4% of the dried fruit, a second group of highly active pigments representing 0.5 to 0.75% of the dried fruit has been separated. Chromatography has revealed this group to contain at least eight different components, three of which preponderate in quantity.

Experimental

Methods. Mature fruits from the osage orange tree were collected, sliced, and set in a forced-air oven at 80°C. until thoroughly dry, then stored at room temperature. Before extraction, the material was ground to pass a 1-mm. sieve.

Antioxidant potencies were determined in oven tests at 100°C. with bleached and deodorized lard,[2] low in antioxidants. This lard was received in 25-lb. lots and was stored in 2-qt. glass containers at −18°C. The antioxidant material to be tested was dissolved in chloroform or ethanol, and an appropriate aliquot was added to 10 g. of the lard in a 9-cm. Petri dish. The same amount of solvent was added to a second 10-g. of lard (control). The solvent was evaporated on a steam table, the dishes were covered and placed in an oven equipped with a circulating fan (100 ± 1°C.). A 0.2-g. sample was withdrawn periodically for a peroxide determination, method of Wheeler (4) modified for small samples. At a peroxide number of *ca.* 20 organoleptic evidence of rancidity was generally observed.

Comparison of Solvents for Extracting Antioxidants from the Osage Orange Fruit. To compare the relative effectiveness of different solvents in concentrating the active substances from the plant material, a series of 50-g. samples of the dried meal was extracted in a Soxhlet type of extractor (Table I). Although ethyl acetate, trichloroethylene, and chloroform produced extracts with the highest activity per gram of extract, methanol was the most effective of the solvents tried in extracting the total activity of the osage orange powder.

TABLE I

Comparison of Solvents for Extracting the Antioxidants

Solvent	Yield[a]	Induction period of lard (10 g.) containing	
		100 mg. of solvent-free extract	Total extract from 1 g. of powder
	%	*hrs.*	*hrs.*
Petroleum ether	12.8	50	50
Acetone	22.8	110	175
Carbon tetrachloride	20.4	110	210
Ethyl acetate	23.9	140	210
Ethyl ether	25.5	115	200
Trichloroethylene	18.2	155	230
Chloroform	21.8	155	230
Methanol	51.8	110	350

[a] Percentage, by weight, of total osage orange powder extracted.

Antioxidant Properties of Pomiferin and Osajin. Each of the two compounds was prepared in quantity and purified by recrystallizing twice from xylene and finally from alcohol. The melting points, pomiferin 200° and osajin 189° (uncorrected), agreed well with literature values reported by Wolfrom and coworkers (6).

[1] Journal Paper No. 913 of the Purdue University Agricultural Experiment Station. The subject-matter of this paper was undertaken initially in cooperation with the Committee on Food Research of the Quartermaster Food and Container Institute for the Armed Forces. The opinions or conclusions contained in this report are those of the authors. They are not to be construed as necessarily reflecting the views or endorsements of the War Department.

[2] Generously prepared and supplied by Swift and Company, Chicago, Ill.

Stability curves obtained by plotting peroxide values *vs.* time were similar to those reported for other well known antioxidants (Figure 1). Typical prooxidant action induced by excessive levels was observed with pomiferin in concentrations greater than 0.2%. However peroxide increments occurred stepwise rather than continuously (Figure 1). This unusual phenomenon was observed repeatedly with crude preparations containing pomiferin as well as with the pure compound. Its significance has not been established.

The effectiveness of pomiferin as an antioxidant for lard was approximately one-half to two-thirds that of NDGA at levels of 0.05% or below and about three-fourths at levels of 0.10% or above. The prooxidant effect appeared at higher levels with pomiferin than with NDGA. With citric, phosphoric, or ascorbic acid, pomiferin exhibited a synergistic response typical of other antioxidants. Citric acid, the most effective, increased the induction period five-fold when added at 0.05% to a .05% level of pomiferin. The prooxidant effect disappeared in the presence of a synergist.

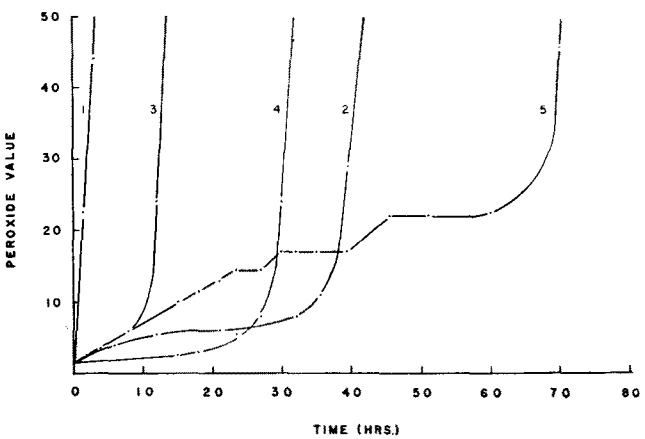

FIG. 1. Peroxide development in lard containing pomiferin.
1. Control
2. 0.10% NDGA
3. 0.05% pomiferin
4. 0.10% pomiferin
5. 0.50% pomiferin

Similar studies with osajin showed this compound to be quite low in antioxidant activity; a level of 0.8% increased the induction period only 2 hrs. over that of a control sample, and citric acid or other synergists did not enhance activity.

Synergistic Properties of the Crude Extracts. The amount of pomiferin present was sufficient to account for only 20–25% of the total activity of the crude extracts. A search was made therefore for other primary antioxidants and/or synergists to account for the unexplained activity. A portion of meal was extracted, first with chloroform, and then with methanol. The chloroform and methanol extracts were then examined for both antioxidant and synergistic activity.

The chloroform extract (50 ml.) was shaken successively with four 50-ml. aliquots of 4% sodium bicarbonate solution, washed twice with 50 ml. of water, and dried over sodium sulfate. Stability tests on the extract before and after this treatment (Table II) indicated that synergism, at least from the usual acidic-type compounds, was not involved in the chloroform-soluble substance.

Methanol extraction yielded an additional 25% of material, which was then tested with lard (Table II). The results showed the methanol extract inactive as a primary antioxidant; however it had active synergistic properties when combined with either the chloroform extract or with crystalline pomiferin. This synergistic action increased the contribution of pomiferin to approximately 75% of the overall activity of the crude extracts.

TABLE II

Antioxidant Properties of a Crude Chloroform Extract and a Subsequent Methanol Extract (Following Chloroform Extraction)

Preparation	Induction Period
	hrs.
1. Unprotected lard (control)	2
2. 0.20% Chloroform extract	24
3. 0.20% Chloroform extract after $NaHCO_3$ treatment	24
4. 0.20% Methanol (chloroform insoluble) extract	2
5. 0.20% No. 2 + 0.20% No. 4	38
6. 0.05% Pomiferin	13
7. 0.05% Pomiferin + 0.05% No. 4	24
8. 0.05% Pomiferin + 0.40% No. 4	53

Isolation of Total Primary Antioxidants. To isolate the active components, other than pomiferin, in the chloroform extracts the technique employed by Wolfrom and Mahan (7) to separate pomiferin from osajin was used. In this separation, insoluble lead salts are formed with *ortho di*-hydroxy compounds, not with *mono*-hydroxy compounds. One kg. of the dried, ground meal was extracted thoroughly with 4 liters of methanol in a continuous extractor (Soxhlet). The extract was cooled and filtered to remove particles of meal which had carried over, and a saturated solution of neutral lead acetate in methanol added until precipitation was complete. The mixture was allowed to stand overnight at room temperature, then filtered; both precipitate and filtrate were saved to prepare Fractions A and B. The precipitate was washed twice with methanol and dissolved in 500 ml. of boiling glacial acetic acid. This solution was poured slowly, with vigorous stirring, into 3 liters of ice and water, and held at 5°C. overnight. The insoluble phenolic compounds were filtered off, washed with cold water, and air-dried. The total yield from one kilogram of the meal was 46 g. (Fraction A).

The methanol filtrate and wash liquors containing the soluble lead salts were concentrated to 1,500 ml., and the excess lead was precipitated with H_2S. The filtrate, after removal of the PbS, was concentrated to 1 liter; an aliquot evaporated to dryness showed the total weight of this fraction (Fraction B) to be 460 g.

Stability tests on Fractions A and B (Table III) indicated that only A contained primary antioxidants. This fraction contained the pomiferin; however the stability imparted to lard was greater than that found with an equal amount of crystalline pomiferin. Since activity of the fraction was not reduced by washing a chloroform solution with 4% $NaHCO_3$ (Table III), the additional activity was not due to acidic synergists.

Chromatography of Fraction A. Fraction A was dissolved in benzene and chromatographed on a pres-

TABLE III

Stabilizing Effect, in Lard, of the Fractions Separated by Neutral Lead Acetate

Preparation	Induction Period
	hrs.
1. Unprotected lard (control)	2
2. 0.5% Fraction B (soluble)	2
3. 0.05% Fraction A (insoluble)	16
4. 0.20% Fraction A	60
5. 0.20% Fraction A after $NaHCO_3$ wash	62

surized (air, 1 atm.) column (20 x 100 mm.) of powdered anhydrous $MgSO_4$. The addition of benzene containing 1% ethanol eluted a well-defined band of pomiferin. A second zone, remaining near the top of the column, was eluted with a mixture of equal volumes of benzene and ethanol (Fraction C). Fraction A was thus separable into pomiferin (80–85%, by weight) and an unknown substance, Fraction C (15–20%, by weight). Fraction C was a highly active antioxidant substance, which also responded synergistically with pomiferin (Table IV).

TABLE IV

Effects of Chromatographic Fractions on Stability of Lard

Preparation	Induction Period
	hrs.
1. Unprotected lard (control)	2
2. 0.10% Fraction C	23
3. 0.30% Fraction C	72
4. 0.10% pomiferin	30
5. 0.10% pomiferin + 0.10% Fraction C	82

Subsequent experiments have shown Fraction C to be further separable into a number of components by chromatography on $MgSO_4$ with smaller increments of ethanol to the benzene. Three major and several minor bands were separated on the column with 2% ethanol in benzene, and two of the major bands (Nos. 4 and 6) were eluted from the column with this solvent. These constituted 25 and 10% (by weight) of Fraction C and both were light green in color. A third major band (No. 8), constituting 45% of Fraction C, was eluted with 4% ethanol in benzene; it was reddish-brown in color.

In stability tests with lard all of the three major bands showed strong antioxidant activity. Induction periods were 20 to 50% longer than that produced with an equal amount of pomiferin. Peroxide accumulation during the induction period (prooxidant effect), typical of pomiferin, NDGA, and other primary antioxidants, was also less pronounced (Figures 2).

The characterization of the three additional primary antioxidants has not been completed. These were separated chromatographically but were not obtained in a crystalline state. However their ultraviolet absorption spectra, with maxima at 273 (band 6) and 274.5 mμ (bands 4 and 8), were very similar to those of pomiferin and osajin, suggesting a close structural relationship to these compounds. Each of the three gave a positive ferric chloride test for phenols and a positive Wilson (5) boric test for flavones and iso-flavones.

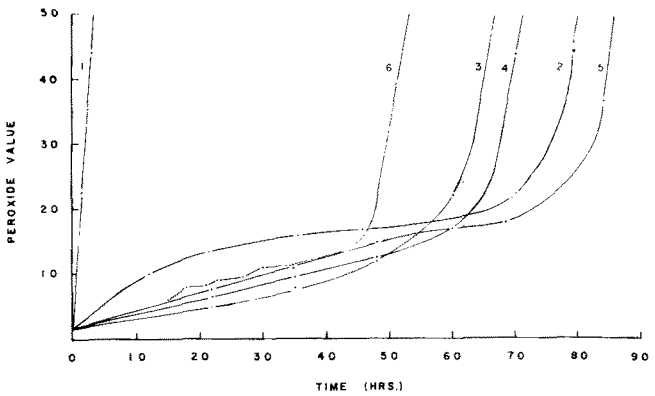

FIG. 2. Peroxide development in lard containing compounds of Fraction C.

1. Control
2. 0.30% NDGA
3. 0.30% band 8
4. 0.30% band 6
5. 0.30% band 4
6. 0.30% pomiferin

Summary

The fruit of the osage orange tree (*Maclua pomifera* [Raf.] Schneider) was shown to contain at least four pigments with antioxidant activity. Pomiferin was present as 3 to 4% of the dry fruit and, as a primary antioxidant, was responsible for 20–25% of the activity exhibited by methanol extracts. An unidentified substance was also present which reacted synergistically with pomiferin, increasing its contribution to the overall activity to approximately 75%. The three new pigments, totalling 0.5% to .75% of the dry fruit, all showed antioxidant activity exceeding that of pomiferin when tested at equal concentrations.

A chromatographic procedure, employing anhydrous magnesium sulfate, was developed and applied successfully in separating mixtures of several iso-flavone pigments.

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Alcoholic Extraction of Vegetable Oils. II. Solubilities of Corn, Linseed, and Tung Oils in Aqueous Ethanol[1]

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IN AN EARLIER communication (1) the need for the study of ethanol as a solvent for vegetable oils was discussed, and complete solubility data on cottonseed, peanut, sesame, and soybean oils in aqueous alcoholic solutions were presented. A literature search revealed no similar data for corn, linseed, and tung oils.

[1] Presented at the Philadelphia fall meeting, American Oil Chemists' Society, October 11, 1955.

Experimental

Unrefined commercially produced oils were used in each case. Their characteristics are given below:

Oil	Acid value	Iodine value (Wij's)	Saponification value
Corn Oil[a]	1.52	120.2	189.7
Linseed Oil	1.48	182.5	191.3
Tung Oil	1.52	168.7	192.5

[a] From wet-milled germs.